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# **Free recall of bound information held in short-term memory is unimpaired by age and education**

**Running title: Memory binding: effects of age and education**

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## **Abbreviations:**

**AD** = Alzheimer's Disease

**STMB** = Short-term memory binding

**MMSE** = Mini-mental State Exam

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## **Free recall of bound information held in short-term memory is unaffected by age and education**

**Running title: Memory binding: effects of age and education**

### **Abstract**

**Objectives:** It has been challenging to identify cognitive markers to differentiate healthy brain aging from neurodegeneration due to Alzheimer's disease (AD) that are not affected by age and education. The Short-Term Memory Binding (STMB) showed not to be affected by age or education when using the change detection paradigm. However, no previous study has tested the effect of age and education using the free recall paradigm of the STMB. Therefore, the objective of this study was to investigate age and education effects on the free recall version of the STMB test under different memory loads.

**Methods:** 126 healthy volunteers completed the free recall STMB test. The sample was divided into five age bands and into five education bands for comparisons. The STMB test assessed free recall of two (or three) common objects and two (or three) primary colors presented as individual features (unbound) or integrated into unified objects (bound). **Results:** The binding condition and the larger set size generated lower free recall scores. Performance was lower in older and less educated participants. Critically, neither age nor education modified these effects when compared across experimental conditions (unbound versus bound features). **Conclusions:** Binding in short-term memory carries a cost in performance. Age and education do not affect such a binding cost within a memory recall paradigm. These findings suggest that this paradigm is a suitable cognitive marker to differentiate healthy brain aging from age-related disease such as AD.

**Key words:** neuropsychology, Learning and Memory, working memory, short-term memory, cognitive aging, neuropsychological tests.

## **Introduction**

Ideal cognitive markers for Alzheimer's disease (AD) should be exempt from the effects of healthy aging, the cultural background and educational level of the assessed individuals (Logie, Parra, & Della Sala, 2015). Identifying cognitive tests with such characteristics has proven challenging. The recently developed short-term memory binding (STMB) test is a promising candidate.

It is well known that the aging process affects significantly some cognitive abilities, such as episodic memory (Nyberg et al., 2003; Rönnlund, Nyberg, Bäckman, & Nilsson, 2005), processing speed (Salthouse, 2000), attention (Verhaeghen & Cerella, 2002) and working memory (Borella, Carretti, & De Beni, 2008), while there are smaller age effects in other abilities, such as semantic memory (Nyberg et al., 2003; Rönnlund et al., 2005) and implicit memory (Jelicic, 1996; Rybash, 1996). However, there is limited knowledge regarding age effects on specific aspects of short-term memory, such as the ability to bind contextual information (relational binding) and intra-item information (conjunctive binding).

It has been demonstrated, in some experimental paradigms, that the ability to bind information in long-term memory declines with age (see Old & Naveh-Benjamin, 2008 for a review), but age effects on STMB tend to be observed only in specific experimental conditions. Age effects have been observed in most studies that require binding contextual information (e.g., item-location: Cowan, Naveh-Benjamin, Kilb, & Sauls, 2006; Fandakova, Sander, Werkle-Bergner, & Shing, 2014; Mitchell et al., 2000), but in some studies this effect has not been observed (Pertzov, Heider, Liang, & Husain, 2015;

Read, Rogers, & Wilson, 2015), probably due to differences in paradigms used. For instance, Mitchell et al. (2000) used a paradigm in which participants should remember objects, locations or object-locations integration of three items (presented separately) after an 8.5 seconds interval. Pertozy et al. (2015), on the other hand, used a task in which participants should remember item-locations integration and, after 1 to 4 seconds interval, should choose which items were presented in the first screen and move it to its previous position. Conversely, age effects have been virtually absent in conjunctive STMB paradigms which require the integration of intrinsic object features (i.e., surface features), such as color and shape (Brockmole, Parra, Della Sala, & Logie, 2008; Isella, Molteni, Mapelli, & Ferrarese, 2015; Killin, Abrahams, Parra, & Della Sala, 2017; Parra, Abrahams, Logie, & Della Sala, 2009; Rhodes, Parra, & Logie, 2016; van Geldorp et al., 2015); but see (Chalfonte & Johnson, 1996). Interestingly, STMB has been reported to be sensitive to the early cognitive changes associated with AD (Della Sala, Parra, Fabi, Luzzi, & Abrahams, 2012; Liang et al., 2016; Parra, Abrahams, Logie, & Della Sala, 2010; Parra, Della Sala, Logie, & Abrahams, 2009).

However, previous studies investigating age effects on STMB have relied on visual recognition assessed via change detection tasks (Brockmole et al., 2008), Experiments 1 and 2; Isella et al., 2015; Killin et al., 2017; Parra, Abrahams, Logie, et al., 2009) or cued recall paradigms (Brockmole et al., 2008, Experiment 3; Brockmole & Logie, 2013; van Geldorp et al., 2015). Whether age spares the ability to freely recall bound features held in short-term memory (STM) remains unexplored.

This is relevant because a free recall version of the STMB test has been used previously to compare performance of healthy and cognitively impaired older adults (Cecchini et al.,

2017; Della Sala et al., 2012; Parra, Abrahams, Fabi, et al., 2009). These studies with clinical samples reported very similar results to those obtained with the visual recognition (change detection) version of the STMB test. That is, AD specifically affected the ability to temporarily hold bound features. However, only the free recall paradigm has been used to compare AD with other dementia types, such as behavioral variant of frontotemporal dementia or Lewy body dementia (Cecchini et al., 2017; Della Sala et al., 2012), showing specificity to AD, while the change detection paradigm was used to compare AD mainly with controls.

Previous studies have calculated the binding cost (BC) as the proportion between remembering isolated features (unbound task) and integrated ones (bound task), showing that it is significantly higher in the AD group when compared to controls (Cecchini et al., 2017). In this study, the control group showed a drop of 11.66%, while the AD group showed a drop of 26.23%. Parra, Abrahams, Fabi et al. (2009) reported data showing 5% drop in the control group and 35% in the AD group, while Della Sala et al. (2012) showed 4% (controls) vs 53% (AD). More recently (Della Sala, Kozlova, Stamate, & Parra, 2016) proposed that this measure holds a reliable classification power to differentiate patients with AD dementia from controls. All these findings suggest that, regardless of the task at hand, the cost of binding may be a good marker of AD dementia. The extent to which such effects were independent of age still needs to be investigated.

As free recall is more dependent on self-initiated processing than recognition or cued recall, it tends to be more sensitive to aging (Danckert & Craik, 2013). Therefore, it is plausible to predict that age effects might be exacerbated in STMB tasks based on free recall, compared to change detection. Although recall declines dramatically with

increasing age, retrieving unified representations (i.e., integrated objects) held in visual STM via recall may be less sensitive to aging than recalling verbal information, as shown by Danckert and Craik (2013). However, an additional challenge of using free recall is that visual stimuli have to be recoded into verbal codes making the task hybrid in terms of visual and verbal STM involvement. Nevertheless, Parra, Della Sala, Logie, & Abrahams (2009) in their Experiment 6 showed that older adults can hold in visual STM arbitrarily paired namable features and can transfer them to verbal STM when the task instructs them to do so.

STMB seems to be insensitive to education and the cultural background of the assessed individual. This suggestion came from a post-hoc analysis using data from a change detection paradigm (Parra et al., 2011). In that study, Parra et al. (2011) investigated the impact of sporadic and familial AD in samples recruited in Scotland (sporadic AD – study 1) and in Colombia (familial AD – study 2). Healthy controls were also recruited in both countries. The means in the STMB test from healthy controls in the different samples were comparable, despite age and education differences. There have been no studies investigating this hypothesis directly (but see (Della Sala et al., 2016) regarding cultural diversity and age effects).

Therefore, the objective of the present study was to investigate if the ability to bind information in STM, based on a free recall task, is affected by age and education. However, age and education effects may be influenced by task demands. For instance, Brockmole et al. (2008, Experiment 1), reported that the number of objects in the display affected older adults' performance more significantly than that of younger adults, suggesting that memory load might magnify the effects of age. Thus, in the present study,

age and education effects were tested in the presence of low (four features) and high (six features) memory load. We predicted that age and education would not affect the ability to bind surface features, and, therefore, that the interaction between age and experimental condition (unbound and bound features) and between education and experimental condition would not be significant. We also predicted that a higher memory load would carry comparable costs in experimental conditions across age and education groups.

## **Methods**

### **Participants**

A group of 126 healthy adults were invited to voluntarily participate in a memory study. Of these, 30 participants were university students and 30 were seniors who participated in Third Age University programs. The remaining participants were recruited from a senior center in the same city. Older participants were independent community dwelling individuals who regularly take part in activities offered at the university or at the center. Participants were recruited while taking part in activities or classes by the researchers who explained the general aims of the study and procedures. If they were interested, later they received a phone call to book an interview. Inclusion criteria required participants to have cognitive scores within the normal range and to be in good self-reported health. All participants gave informed consent to take part in the study, which was approved by the relevant Ethics Committees (protocol number 16627413.0.0000.0068).

To investigate age effects on STMB, the sample was divided into five age groups, one of young adults (18-25) and four of older adults (60-64, 65-69, 70-74, and 75 years and older). To investigate education effects on STMB, older adults were divided into five



education bands (No education, 1-4 first level primary school, 5-8 second level primary school, 9-11 high school, and 12 and higher years of education). Sample sizes are displayed in Tables 1 and 2. Participants were regarded as having no education when they reported having received no formal schooling or having completed less than one year of education. For the analysis of the education effect, we focused only on the older group as our study was aimed at investigating the validity of the novel STMB to assess populations at risk of dementia.

### **Instruments and procedures**

Participants completed a socio-demographic and health questionnaire and cognitive tests to ascertain normal cognitive status (Young adults: WAIS-III Block Design and Vocabulary tests Nascimento, 2004; Wechsler, 1997) (Older adults: Mini Mental State Examination – MMSE Brucki, Nitrini, Caramelli, Bertolucci, & Okamoto, 2003; Folstein, Folstein, & McHugh, 1975); and the Addenbrooke's Cognitive Examination Revised - ACE-R Carvalho, Barbosa, & Caramelli, 2010; Mioshi, Dawson, Mitchell, Arnold, & Hodges, 2006). Cut off scores to determine normal cognitive performance in the MMSE were 17 points for illiterate participants, 22 for those with 1 to 4 years of schooling, 24 for those with 5 to 8 years and 26 for those with 9 or more years of schooling (Brucki et al., 2003). ACE-R scores were also used to ascertain normal cognitive performance, according to Brazilian normative data (Amaral-Carvalho & Caramelli, 2012).

From the conjunctive STMB paradigms previously reported (Della Sala et al., 2012; Parra, Abrahams, Fabi, et al., 2009) we chose two conditions, the unbound features and bound features conditions. The rationale behind this selection is the notion that the

unbound features condition might represent a better baseline condition to assess binding costs than single-feature conditions (i.e., Color or Object Only). The unbound features condition contains the same number and type of features as the bound condition, yet, the only difference between them is the need to bind them together in the latter.

### **Initial screening for color blindness and inability to bind in perception**

A personal computer running an E-prime script (Psychological Software Tools, Pittsburgh, PA) generated for the study was used to screen for color blindness and perceptual binding deficits, and to apply the STMB tasks. To rule out color blindness, participants were assessed with the simplified five-plate Ishihara test (Ishihara, 2008). Two errors or more were set as exclusion criterion. Five of the participants recruited for the present study demonstrated color blindness. In order to ascertain that color blindness was not affecting results, we analyzed the data excluding these five participants, who had missed two trials in the Ishihara test. After the Ishihara test, participants were given the Perceptual Binding Test (see Parra et al., 2010). This task assesses the ability to form shape-color bindings in perception, a function necessary to transfer bound information to STM. Participants had to score 80% or above (out of 16 trials) to proceed to the STM task. In the present study, participants were not excluded due to the inability to bind. After that, participants were given sample trials of the change detection STMB. This gave the experimenter a chance to explain the paradigm and evaluate if the participant could complete them.

## **Free recall STMB**

The stimuli for this task consisted of one set of 11 nameable colors (red, blue, green, brown, orange, yellow, purple, grey, turquoise, pink and black) and another with 11 nameable objects (bed, apple, banana, bell, shoe, car, book, chair, cup, guitar and button). These colors and objects were used to construct the stimulus arrays as described in a previous study (Parra, Abrahams, Fabi, et al., 2009). At the beginning of the experiment participants were presented with two separate arrays, one consisting of 20 colors and the other consisting of 20 objects. These arrays comprised 11 colors and 11 objects used in the experiment and other 9 colors and 9 objects intermixed within the arrays as distractors. Participants were requested to name the colors and the objects to ensure that they had no problems naming the items used in the experiment. All participants correctly named the displayed objects and offered acceptable color names. Participants then completed two blocks of trials. Each block consisted of a set of trials with two stimuli arrays followed by the same number of trials with three stimuli arrays: free recall with unbound features (6 trials with 2 colors and 2 objects; 6 trials with 3 colors and 3 objects) and free recall with bound features (6 trials with 2 colored objects; 6 trials with 3 colored objects). The study array was presented for 1.5 sec per feature, each color and each object were considered as one feature (6 seconds with 4 features [unbound with 4 items and bound with 2] and 9 seconds with 6 features [unbound with 6 items and bound with 3]). Blocks were counterbalanced across participants (see Figure 1).

***Unbound Features:*** in this condition, the study array consisted of two (or three) colors and two (or three) objects presented as separate entities. Half of the items were colored squares and the other half were line drawings of common objects. All objects and colors

in the array were displayed simultaneously. Participants were given the following instructions: ‘Now we will test your memory for colors and objects. You will see two (or three) colors and two (or three) objects on the screen. You should try to remember as many colors and objects as you can. After these colors and objects disappear, you will have to say aloud all the colors and objects that you have just seen’. The experimenter recorded the responses using a scoring sheet.

***Bound Features:*** in this condition the study array consisted of two (or three) objects filled with a different color each, presented for 1.5 sec per feature. The objects in the array were displayed simultaneously. These colored objects were constructed by randomly combining objects with colors from the two sets always avoiding prototypical object-color pairings (e.g., red apple). During this condition participants were asked: ‘You should try to remember as many colored objects as possible, that is, remember each object together with the color in which it was presented’. The participants should memorize the *combination* of colors and objects, for instance: “red-bed”, or “green-shoe”. A correct response was considered only when the two features (color and object) were recalled together. The experimenter recorded responses using a scoring sheet. Figure 1 presents an illustration of this task.

INSERT FIGURE 1 HERE.

### **Statistical Analyses**

As the groups showed significant difference in age and educational level, we used ANCOVA models to compare the groups. In the STMB tasks, the percentage of correctly

recalled items was used. To test if there was a difference in the proportion between the performance on the Unbound and on the Bound conditions, the binding cost variable was calculated as the percentage of loss in performance observed in the bound condition compared to the unbound condition ( $\text{Binding cost} = 100 - 100 \times (\text{bound/unbound})$ ). Two  $5 \times 2 \times 2$  mixed ANCOVA models were implemented. For the between-subject factor Age, the levels were Young x 60-64 x 65-69 x 70-74 x 75+ years of age, and education was used as covariate. For Education, the levels were 0 x 1-4 x 5-8 x 9-11 x 12+ years of education, and age was the covariate. For both models the within-subject factors were Condition (Unbound versus Bound) and Set Size (4 features versus 6 features). Effect sizes were also calculated. Significance level was set at 0.05. Significant effects and interactions were followed by Bonferroni-corrected post-hoc comparisons.

Considering that the primary aim of the study was to demonstrate the possible lack of an effect of age or education, Bayesian analyses were also carried out, as Bayes Factors can help quantify the evidence for a null effect. Bayesian analyses were carried out for all group comparisons (ANCOVAs and mixed ANCOVAs), using Bayes factor ( $\text{BF}_{10}$ ) to interpret the results. The  $\text{BF}_{10}$  was interpreted as the probability of the alternative hypothesis being correct when compared with the null one. The following criteria were used (Wagenmakers et al., 2018): 0-1: no evidence; 1-3: anecdotal evidence; 3-10: moderate evidence; 10-30: strong evidence; 30-100: very strong evidence;  $> 100$ : extreme evidence. The mixed models were interpreted using the analysis of effects output, in which the models that contain the effect are compared to equivalent models stripped of the effect. The ANCOVA models and mixed models were performed in SPSS v25, and the Bayesian analyses were carried out in JASP v 9.2.0.

## Results

### Age effects

Table 1 presents the means and standard deviations for education, cognitive measures and STMB performance for the age groups, with the ANCOVA comparisons. Older participants (70-74 and 75+) had lower education than the young adults and younger older adults (60-64 and 65-69). The older groups showed worse performance in the tasks with the larger set size, but no difference regarding the binding cost variable.

INSERT TABLE 1 HERE

INSERT FIGURE 2 HERE

The results from the mixed models indicated a significant main effect of Age Group [ $F(4,115)=7,116$ ,  $p<0.001$ ,  $\eta^2=0.198$ ,  $\beta=0.994$ ,  $BF_{10}=1.851e+8$ ] and Set size [ $F(1,115)=78\ 767$ ,  $p<0.001$ ,  $\eta^2=0.407$ ,  $\beta=1.000$ ,  $BF_{10}=6.094e+30$ ], no effect of Condition [ $F(1,115)=0.327$ ,  $p=0.568$ ,  $\eta^2=0.003$ ,  $\beta=0.088$ ,  $BF_{10}=10.250$ ], while the Bayesian analyses showed moderate evidence in favor of the condition effect. The young adults and those who were 60-64 and 65-69 years old outperformed the older participants. In addition, the bound features condition and the larger set size generated lower performance. The Condition x Set size x Age Group interaction was significant [ $F(4,115)=2.825$ ,  $p=0.028$ ,  $\eta^2=0.089$ ,  $\beta=0.755$ ,  $BF_{10}=0.0.149$ ], but with no evidence of significance using Bayesian analyses. There was a significant Set size by Age Group interaction [ $F(4,115)=6.799$ ,  $p<0.001$ ,  $\eta^2=0.191$ ,  $\beta=0.992$ ,  $BF_{10}=2.044e+6$ ], as older

participants (70-74, 75+) had worse performance in set size 6. However, the Condition by Set size interaction was not significant [ $F(1,115) < 0.001$ ,  $p = 0.987$ ,  $\eta^2 < 0.001$ ,  $\beta = 0.050$ ,  $BF_{10} = 11.464$ ], but with strong evidence in favor of the alternative hypothesis in Bayesian analyses. That possibly means that a different Set size did not change significantly how participants performed in the bound and unbound conditions proportionally. In addition, and most importantly, there was not a significant Condition by Age Group interaction [ $F(4,115) = 0.534$ ,  $p = 0.711$ ,  $\eta^2 = 0.018$ ,  $\beta = 0.175$ ,  $BF_{10} = 0.018$ ], which suggests that the older groups were not disproportionately affected by conditions requiring to bind features in STM relative to those requiring to hold them individually, and this was true regardless of memory load.

### **Education effects**

Table 2 presents the means and standard deviations for age and cognitive performance with the sample of older participants divided according to their level of education, with ANCOVA comparisons. The group with no schooling was older and had worse cognitive scores (MMSE, ACE-R and BD) when compared with the group with higher educational level. There were no differences between the education groups for the Unbound with 4 items, Bound with 4 or 6 items, and binding cost variables. Figure 3 presents means and error bars according to education and memory load.

INSERT TABLE 2 HERE.

INSERT FIGURE 3 HERE

The results from the mixed models indicated a significant main effect of Education Group [ $F(4,85)=3.683$ ,  $p = 0.008$ ,  $\eta^2 = 0.148$ ,  $\beta = 0.865$ ,  $BF_{10}=856.928$ ]. There was no effect of Condition [ $F(1,85)=0.223$ ,  $p = 0.638$ ,  $\eta^2 = 0.003$ ,  $\beta = 0.075$ ,  $BF_{10}=2.229$ ]. There was no effect of Set size in traditional analyses [ $F(1,85)=0.103$ ,  $p = 0.749$ ,  $\eta^2 = 0.001$ ,  $\beta = 0.062$ ,  $BF_{10}=1.686e+29$ ], but extreme evidence using Bayesian analyses. Participants with higher education outperformed those with less or no education. Also, the bound condition and set size 6 generated worse performance. The three way interaction Condition x Set size x Education Group was non-significant [ $F(4,85)=1.367$ ,  $p = 0.252$ ,  $\eta^2 = 0.060$ ,  $\beta = 0.409$ ,  $BF_{10}= 0.061$ ]. The interaction between Set Size and Education Group did not reach significance [ $F(4,85)=1.510$ ,  $p = 0.207$ ,  $\eta^2 = 0.066$ ,  $\beta = 0.449$ ,  $BF_{10}=0.343$ ]. The interaction between Condition and Set Size was significant [ $F(1,85)=7.026$ ,  $p = 0.010$ ,  $\eta^2 = 0.076$ ,  $\beta = 0.746$ ,  $BF_{10}=6.639$ ], with moderate evidence for this interaction, as worse performance was observed in the bound condition in the larger set size. Most importantly, the Condition x Education Group interaction was non-significant [ $F(4,85)=1.125$ ,  $p = 0.350$ ,  $\eta^2 = 0.050$ ,  $\beta = 0.340$ ,  $BF_{10}=0.105$ ], which suggested that in older adults education does not disproportionately affect the ability to bind information in STM more that it does for individual features and, as per age, this was true regardless of memory load.

## Discussion

The present study was aimed at investigating age and education effects on STMB in the free recall modality, with varying memory loads (four and six bound or unbound features). Presently, 22% of adults 65 and older are illiterate (141 million individuals) (UNESCO, 2017) and the diagnosis of pathological cognitive changes in this group is challenging as screening tests are influenced by educational level. Therefore, identifying tests that are exempt from education effects is vital.



The results indicated that performance was lower in participants with higher age and lower education. In addition, the bound feature condition and the larger set sizes generated lower scores in STMB free recall. Nevertheless, evidence originated from ANCOVA analyses and Bayesian statistics allows us to claim that neither age nor education affects the ability to bind intrinsic features into unique objects. This is supported by the findings that age and education groups did not differ in the binding cost variable, and by the mixed models that showed that neither age nor education interacted significantly with condition (bound and unbound). These results were also supported by Bayesian statistics which quantify the evidence for a null effect. Therefore, present findings suggest that age and education did not differentially affect performance across experimental conditions (unbound vs bound) even when the task was more challenging (set size with 6 features).

It is necessary to highlight that results indicated that binding in STM carries a cost, as performance declines from the unbound to the bound condition. Yet, the lack of significant interactions between age or education and the experimental conditions (unbound vs bound) indicate that age and education do not affect such a binding cost within a memory recall paradigm.

### **Lack of specific age effects on free recall STMB**

These results are in agreement with previous reports which suggested a lack of age effect on binding cost using the change detection or cued recall paradigms (Brockmole et al., 2008; Isella et al., 2015; Killin et al., 2017; Parra, Abrahams, Logie, et al., 2009; Rhodes et al., 2016). The results presented here are novel, as they showed no age effects on

binding functions responsible for holding conjunctions of features in STM. Such age effects had not been investigated previously using a free recall task. Also, our results lend support to the hypothesis that the deficits observed in samples with AD using the free recall STMB test (Cecchini et al., 2017; Della Sala et al., 2012; Liang et al., 2016; Parra et al., 2010; Parra, Della Sala, et al., 2009) are likely due to neurodegeneration and not to brain aging. Moreover, to our knowledge, this is the first study documenting among healthy seniors the presence of preserved binding functions in aging operating through a retrieval function (i.e., recall), known to decline with age much more pronouncedly than recognition (Danckert & Craik, 2013). The fact that older participants showed worse overall performance than the younger group, but did not show an increased deficit to bind information is relevant as, when identified, binding deficits may therefore signal neuropathology, especially AD. In addition, present findings confirm that some components of visual STM may remain preserved in aging, even when memory load is increased.

Considering the present findings and those from previous studies with the change detection paradigm, there is evidence to suggest that intra-item feature binding ability remains age insensitive regardless of the retrieval strategy used to assess this function. Taken together these results suggest that the process of forming and holding objects' identity is spared by healthy aging (see Parra, 2017).

### **Lack of specific education effects on free recall STMB**

The present study tested ad-hoc the hypotheses that education would not differentially impact performance on the free recall version of the STMB test. This hypothesis was

motivated by the post-hoc findings by Parra et al. (2011) using data from a change detection task collected from healthy controls with different schooling levels from Colombia and Scotland and patients with different variants of AD (familial and sporadic). The observed lack of education effects on the binding cost has important clinical implications as it suggests the test may be used for early AD detection among seniors with diverse cultural and educational backgrounds. Education has proved to be an important confounding variable whose control not always yields the expected outcomes (Brucki, Mansur, Carthery-Goulart, & Nitrini, 2011; Mungas et al., 2010).

### **Set size effects on free recall STMB**

In the present study, memory load was manipulated to create an easy and a more challenging version of the test. Set size significantly interacted with age, but not with education (although Bayesian statistics suggested a significant interaction). These results indicate that under challenging task conditions, age effects and most likely education effects on overall performance are amplified. The fact that there was a significant Set size and Condition interaction in the ANCOVA comparing educational bands denotes that the cost of binding is higher in the context of a more challenging task. Nevertheless, performance across experimental conditions did not dissociate across age or educational levels, thus reinforcing the lack of sensitivity of these functions to these demographic factors. Indeed, the validity of this methodology as a transcultural cognitive marker of AD has been recently suggested (Della Sala et al., 2016).

The fact that the sample was recruited in a low to middle income country is also relevant, as it suggests STMB findings are not region or culture specific. In fact, the lack of

education effects on the binding cost may be of particular interest to low income countries where cognitive instruments tend to generate an elevated number of false positive cases during the screening for dementia symptoms, an outcome largely driven by low education (e.g., Brucki & Nitrini, 2014; Parra, 2014). Cognitive markers free from education effects may also be highly relevant to countries interested in the early diagnosis of dementia among immigrants with diverse backgrounds (Mungas, Reed, Farias, & DeCarli, 2009; Nielsen, Vogel, & Waldemar, 2012).

In sum, the present investigation adds to the array of evidence confirming that STMB functions are not differentially affected by age or education. We have expanded this evidence to a new retrieval modality (i.e., recall) and to a population with particular demographic characteristics, such as low or no education. The free recall STMB test may aid in the characterization of cognitive aging trajectories in populations with diverse educational background.

Limitations of the current investigation include the fact that participants did not undergo a full neuropsychological exam and the sample might have included participants with mild cognitive deficits. However, such a caveat could affect all age groups, but specially the older and less educated groups, since being less educated and older increase the risk of cognitive deficits. Thus, this would increase the probability of finding an effect of age and education in STMB tasks. In addition, there were education and age differences among the compared sub-groups. We argue that the fact that older adults had significantly lower education went against study hypothesis, and that even when facing a double jeopardy (higher age and lower education) seniors with these characteristics did not show

a specific binding deficit. As a merit of the study, we cite that the sample included seniors with no schooling, who are less frequently described in aging studies.

Future studies should relate STMB tasks to AD biomarkers, especially beta amyloid and tau, to further test the hypothesis of the specificity of binding deficits as a cognitive marker for AD. In addition, future studies could explore the relationship between free recall STMB scores and neuroimaging parameters (structural and functional) to allow a better understanding of brain regions involved with conjunctive binding.

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## Tables

**Table 1.** Means and standard deviations for education and cognitive scores with the sample divided into groups according to age (N=126).

	Young (n = 30)	60 – 64 (n = 20)	65 – 69 (n = 33)	70 – 74 (n = 21)	75 + (n = 17)	p- value	BF <sub>10</sub>
Age	20.07(2.23) <sup>bcd</sup>	61.70(1.78) <sup>acde</sup>	66.52(1.25) <sup>abde</sup>	71.67(1.16) <sup>abce</sup>	79.18(3.38) <sup>abcde</sup>	<0.001	2.094e+116
Education	12.50(1.17) <sup>de</sup>	10.55(3.75) <sup>de</sup>	9.45(5.40) <sup>d</sup>	3.95(5.56) <sup>abc</sup>	5.53(6.20) <sup>ab</sup>	<0.001	2.922e+6
MMSE	-	27.85(2.06)	26.36(3.05)	24.67(3.65)	24.94(3.05)	0.397	0.171
ACE-R	-	85.63(16.16)	77.52(21.50)	66.05(17.70)	68.94(16.34)	0.555	0.154
BD	39.73(14.69) <sup>bcd</sup>	27.45(9.68) <sup>a</sup>	26.15(11.34) <sup>a</sup>	16.95(11.01) <sup>a</sup>	17.16(7.44) <sup>a</sup>	<0.001	110.4
Vocabulary	28.47(8.96)	-	-	-	-	-	-
Unbound 4	94.31(8.08) <sup>e</sup>	91.25(7.99) <sup>e</sup>	89.52(8.85) <sup>e</sup>	82.54(14.04)	76.72(21.65) <sup>abc</sup>	0.010	4.942
Bound 4	92.22(9.52)	90.42(13.32)	87.88(11.62)	82.54(8.70)	78.92(13.22)	0.121	0.473
BC 4	2.23(5.22)	0.62(14.57)	1.61(10.86)	-1.99(14.89)	-11.93(37.17)	0.168	0.458
Unbound 6	91.20(9.73) <sup>cde</sup>	83.47(8.81)	77.61(11.68) <sup>a</sup>	67.86(13.42) <sup>a</sup>	69.12(14.73) <sup>a</sup>	<0.001	181.9
Bound 6	87.59(13.66) <sup>cde</sup>	80.00(12.67) <sup>de</sup>	73.06(13.47) <sup>a</sup>	58.47(14.87) <sup>ab</sup>	61.11(14.96) <sup>ab</sup>	<0.001	29704.830
BC 6	3.99(11.11)	4.18(10.88)	3.74(22.97)	13.09(19.76)	9.84(20.13)	0.069	0.232

Note. MMSE = Mini-Mental State Examination; ACE-R = Addenbrooke Cognitive Examination Revised total score.

BD = Block Design test (WAIS-III). Vocabulary = Vocabulary test (WAIS-III). BC = Binding cost. Letters denote significant group differences in multiple comparisons: a = differ from “young” group; b = differ from “60 - 64” group; c = differ from “65 - 69”; d = differ from “70 - 74”; e = differ from “75 +”. Significance level was set at 0.05. There were missing cases for ACE-R (1 case for 60-64, 1 case for 75+), and Block Design (2 cases 60-64). Analyses were carried out using education as covariate for the STMB tasks and Binding Cost variable.

**Table 2.** Means and standard deviations for age and cognitive scores with the sample divided into groups according to education (n=91).

	No schooling (n = 17)	1 – 4 (n = 19)	5 – 8 (n = 13)	9 – 11 (n = 20)	12 + (n = 22)	p-value	BF <sub>10</sub>
Age	72.00(4.72) <sup>cd</sup>	73.95(6.35) <sup>cde</sup>	64.46(4.93) <sup>ab</sup>	66.2(2.93) <sup>ab</sup>	67.68(6.48) <sup>b</sup>	<0.001	9010
Education	0(0.0) <sup>bcde</sup>	2.68(1.11) <sup>acde</sup>	7.15(1.28) <sup>abde</sup>	10.88(0.45) <sup>abce</sup>	15.41(1.18) <sup>abcd</sup>	<0.001	2.787e+64
MMSE	21.82(2.94) <sup>bcde</sup>	24.58(2.04) <sup>acde</sup>	27.08(2.22) <sup>ab</sup>	28.00(1.69) <sup>ab</sup>	28.14(1.78) <sup>ab</sup>	<0.001	1.975e+11
ACE-R	55.06(8.68) <sup>cde</sup>	68.58(10.46) <sup>e</sup>	81.92(7.38) <sup>a</sup>	75.20(28.62) <sup>ae</sup>	92.81(3.723) <sup>abd</sup>	<0.001	1.488e+6
BD	10.53(3.68) <sup>bde</sup>	16.58(5.54) <sup>ade</sup>	20.00(6.26) <sup>de</sup>	30.84(8.26) <sup>abc</sup>	32.33(9.00) <sup>abc</sup>	<0.001	9.890e+11
Unbound 4	80.15(14.02)	80.04(21.76)	89.10(7.89)	89.79(8.05)	89.96(10.18)	0.634	0.123
Bound 4	78.43(8.36)	82.02(12.51)	89.74(12.34)	86.25(13.04)	90.91(11.18)	0.111	0.860
BC 4	-0.17(16.98)	-10.72(34.60)	-1.35(15.72)	3.94(11.87)	-1.53(11.07)	0.584	0.155
Unbound 6	62.75(10.89) <sup>de</sup>	67.98(14.33) <sup>e</sup>	77.56(8.82)	81.53(9.25) <sup>a</sup>	83.33(10.07) <sup>ab</sup>	<0.001	2068.223
Bound 6	59.81(16.02)	63.16(16.27)	73.08(12.39)	70.83(13.23)	77.02(16.02)	0.105	0.728
BC 6	3.60(25.28)	6.08(19.78)	5.90(11.77)	13.56(13.72)	6.24(23.38)	0.266	0.132

Note. MMSE = Mini-Mental State Examination; ACE-R = Addenbrooke Cognitive Examination Revised; BD = Block Design test (WAIS-III). Vocabulary = Vocabulary test (WAIS-III). BC = Binding cost. Letters denote significant group differences in multiple comparisons: a = differ from “no schooling” group; b = differ from “1 – 4” group; c = differ from “5 – 8”; d = differ from “9 – 11”; e = differ from “12 +”. Significance level was set at 0.05. There were missing cases for ACE-R (1 case 5-8, 1 case 12+) and Block Design (1 case 9-11, 1 case 12+). Analyses were carried out using education as covariate for the STMB tasks and Binding Cost variable.

**Figures legends**

**Figure 1.** Free recall STMB test in the unbound and bound conditions with six features.

**Figure 2.** Means and standard errors (error bars) for the STMB according to age and memory load.

**Figure 3.** Means and standard error bars for the STMB according to education and memory load.